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ANTIOXIDANT SCREENING AND POLYPHENOLS PROFILING OF *IPOMOEA ASARIFOLIA* (DESR.) ROEM. & SCHULT (CONVOLVULACEAE)

MEDA Nâg-Tiero Roland[1*], COMPAORE Moussa[2], BANGOU Mindiédiba Jean[1], VLASE Laurian[3], KIENDREBEOGO Martin[2], OUEDRAOGO Anicet Georges[1]

[1]Laboratory for Research and Education in Animal Health and Biotechnology, University of Nazi BONI, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso.

[2]Laboratory of Applied Biochemistry and Chemistry, University of Ouaga I, Professor Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso.

[3]Department of Pharmaceutical Technology and Biofarmceutics, University of Medicine and Pharmacy «Iuliu Hatieganu», Faculty of Pharmacy, 4 Pasteur Street, Cluj-Napoca, Romania.

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***Corresponding Author**
MEDA Nâg-Tiero Roland
Laboratory for Research and Education in Animal Health and Biotechnology, University of Nazi BONI, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso.

ABSTRACT

Ipomoea asarifolia, native to tropical America is widely used in Africa traditional medicine for the treatment of various diseases. This plant is well used in Burkina Faso folk medicine to manage malaria, convulsions and rheumatism. For that, the organic fractions of *I. asarifolia* hydroacetonic extract have been screened for their antioxidant activity and polyphenols profile. The ferric reducing antioxidant power (FRAP), the ABTS radical cation scavenging capacity and the free radical scavenging capacity (DPPH) were used to evaluate the antioxidant abilities. The total phenolics and flavonoids were quantified using Folin-ciocalteu and aluminium chloride tests, respectively and the phenolic compounds were analyzed by HPLC-MS. The results obtained have demonstrated that the ethyl acetate fraction exhibits a remarkable antioxidant according to the three methods. In this study gentisic, *p*-coumaric and ferulic acids and hyperoside, isoquercitrin, quercitrin and quercetol were identified. Reported chlorogenic and caffeic acids were also found. These finding demonstrated that *I. asarifolia* extracts contained some antioxidant compounds which could support the traditional uses.

KEYWORDS: Polyphenols; Antioxidant activity*; Ipomoea asarifolia*; HPLC-MS.

1. INTRODUCTION

Ipomoea asarifolia (Desr.) Roem. & Schult is a hairless, succulent perennial weed of the Convolvulaceae family that grows in hydromorphic soils in low lying and inland valleys, streams, and river banks. It is native to tropical America but now it is found throughout West Africa from Cameroun to Senegal, Mali and Burkina Faso. [1, 2]

In Senegal, *I. asarifolia* is used traditionally for various gynecological purposes, ophthalmias, neuralgia, headaches, arthritic and stomach pains while in northern of Nigeria, the leaf poultice is applied to guinea worm sores. [2] The leafy stems are used in Burkina Faso to treat malaria, teething children, convulsions, while the roots are used to treat rheumatism. [1]

Pharmacological and phytochemical investigations have already conducted on *I. asarifolia* extracts. The stem bark, leaves and root bark of this plant have been shown to possess significant antioxidant properties. [3] The anti-inflammatory, antinociceptive and analgesic properties have been already documented. [2, 4, 5] It's also demonstrated that *I. asarifolia* leaves have potent hepatoprotective activity against CCl_4-induced hepatic damage in rats. [6] Preliminary phytochemical screening have shown the presence of anthraquinones, saponins, tannins, triterpenes, flavonoids and alkaloids. [7] The flowers of this plant are a rich source of anthocyanins. [8] Recently, phenolic compounds (caffeic acid, chlorogenic acid and rutin) [5] and news indole diterpenes [9] have been reported.

Due to the importance of *I. asarifolia* in the traditional medicine of Burkina Faso, it is necessary to prove the scientific knowledge for justifying its uses.

The present study aims to evaluate the antioxidant activity of the organic fractions form *I. asarifolia* hydroacetonic extract and to determine the polyphenols profile of the most active antioxidant fraction.

2. MATERIAL AND METHODS

2.1. Chemicals and reagents

All reagents were of analytical grade. Folin-Ciocalteu reagent, NaH_2PO_4, Na_2HPO_4, sodium carbonate, aluminium trichloride and gallic acid were from Sigma-Aldrich Chemie (Steinheim, Germany). 2,2-Diphenyl-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonate) ABTS, trichloroacetic acid, potassium persulfate,

acetonitrile, methanol, acetone, *n*-hexane, *n*-butanol, dichloromethane and ethyl acetate were from Fluka Chemie (Buchs, Switzerland). Potassium hexacyanoferrate [$K_3Fe(CN)_6$] was from Prolabo (Paris, France) and ascorbic acid was from Labosi (Paris, France). Caftaric acid was from Dalton (Toronto, ON, Canada), gentisic acid, ferulic acid, sinapic acid, patuletin, luteolin from Roth (Karlsruhe, Germany), caffeic acid, chlorogenic acid, *p*-coumaric acid, hyperoside, isoquercitrin, rutoside, myricetol, fisetin, quercitrin, quercetol, kaempferol and apigenin were from from Sigma (St. Louis, MO, USA)

2.2. Plant materials

The whole plant of *Ipomoea asarifolia* was collected in May 2011 at Gampella, 25 Km east from Ouagadougou (Burkina Faso). The plant identification was done by Prof. Millogo-Rasolodimby and the Voucher specimen (MR_05) was deposited in the OUA herbarium of the CIB (Centre d'Information sur la Biodiversité), UFR/SVT of the University of Ouagadougou.

2.3. Extraction and fractionation

The aqueous acetone extract was obtained using 50 g of dried and powdered from whole plant.[10] The *n*-hexane fraction (*n*-HF), dichloromethane fraction (DCMF), acetonitrile fraction (ACN), ethyl acetate fraction (EAF), methanol fraction (MeOHF) and *n*-butanol fraction (*n*-BuOHF) were obtained after sequential liquid-liquid extraction. For HPLC-MS analysis, the ethyl acetate fraction was used to prepare non-hydrolysed sample (NHS) and hydrolysed sample (HS) as describe by Meda et *al*.[11]

2.4. Antioxidant activity determination

The antioxidant activity determination using the iron (III) to iron (II) reduction (FRAP) assay, the $ABTS^{\bullet+}$ (2,2'-azinobis-3- ethylbenzothiazoline-6-sulphonate) radical cation and the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging methods were done as described by Meda *et al*. [10] Data were expressed in µmol ascorbic acid equivalent (AAEAC) per g of fraction, µmol trolox equivalent (TEAC) per g of fraction and µmol of quercetin equivalent (QEAC) per g of fraction, respectively.

2.5. Phytochemical analysis

2.5.1 Determination of total phenolics and total flavonoids

Total phenolics and total flavonoids of the fractions were determined using Folin-Ciocalteu and aluminium trichloride methods. The experimental procedure was fully described in Meda *et al.* [10]

2.5.2. HPLC-MS analysis of polyphenols

The ethyl acetate fraction was screened for 18 phenolic compounds analyzation using Agilent 1100 HPLC Series system. The Apparatus and chromatographic conditions have been described. [11, 12] Calibration curves in the 0.5–50 µg/mL range with good linearity (R^2 > 0.999) for a five point plot were used to determine the concentration of polyphenols in the plant samples. The Agilent ChemStation (vA09.03) and DataAnalysis (v5.3) software were used for the acquisition and analysis of chromatographic data. [12]

2.6. Statistical Analysis

The data were expressed as Mean ± Standard deviation (SD) of three determinations. Statistical analysis (ANOVA with a statistical significance level set at p<0.0001 and linear regression) was carried out with XLSTAT 7.1.

3. RESULTS

3.1. Antioxidant activity

The ferric reducing antioxidant power, the $ABTS^{\bullet+}$ radical cation and the free radical scavenging capacities results are showed in "Fig. 1(A, B & C)".

The ability of the fractions from *I. asarifolia* aqueous acetone extract to reduce Fe(III) to Fe(II) was ranged from 169.828 to 2559.512 µmol AAE/g of fraction "Fig. 1 (**A)**". The EAF (2559.512 µmol AAE/g) reduce twice more Fe(III) to Fe(II) than the DCMF (1314.386 µmol AAE/g). A weak activity was found with the *n*-HF (169.828 µmol AAE/g).

The "Fig 1. (**B)**" indicated the ABTS radical cation scavenging capacity of *I. asarifolia* fractions. The fraction obtained from the EA solvent showed the strongest activity (363.963 µmol TE/g of fraction). Interesting activities are also obtained in the DCMF and the ACNF. *n*-HF presented the lowest ABTS radical cation scavenging capacity.

The EAF (212.455 µmol QE/g of fraction) and the DCMF (213.106 µmol QE/g of fraction) have scavenged more the free radicals than the MeOHF (176.022 µmol QE/g of fraction). No activity was found with the fraction obtained from *n*-hexane "Fig 1. (**C)**".

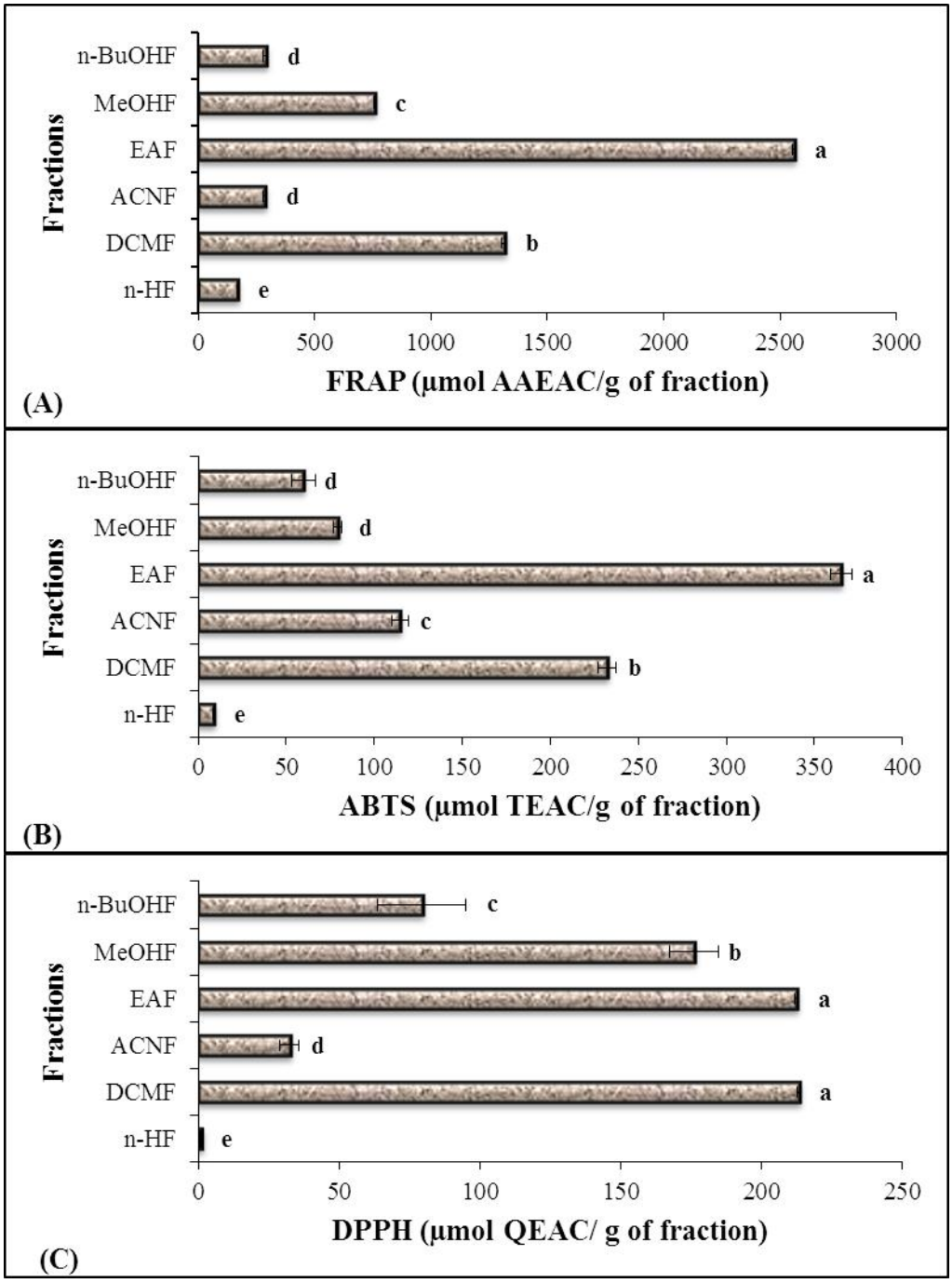


Figure 1: Antioxidant activities obtained using the FRAP (A), ABTS (B) and DPPH (C) methods on organic fractions. *n*-HF: *n*-hexane fraction; DCMF: dichloromethane fraction; ACNF: acetonitrile fraction; EAF: ethyl acetate fraction; MeOHF: methanol fraction; *n*-BuOHF: *n*-butanol fraction. Values are mean ± SD (n = 3). Different letters indicate significant difference ($p < 0.0001$)

3.2. Phytochemical screening

The total phenolics per g of fractions was ranged from 22.367 to 598.327 mg GAE and that of the total flavonoids was 0 to 22.468 mg EQ "Table 1".

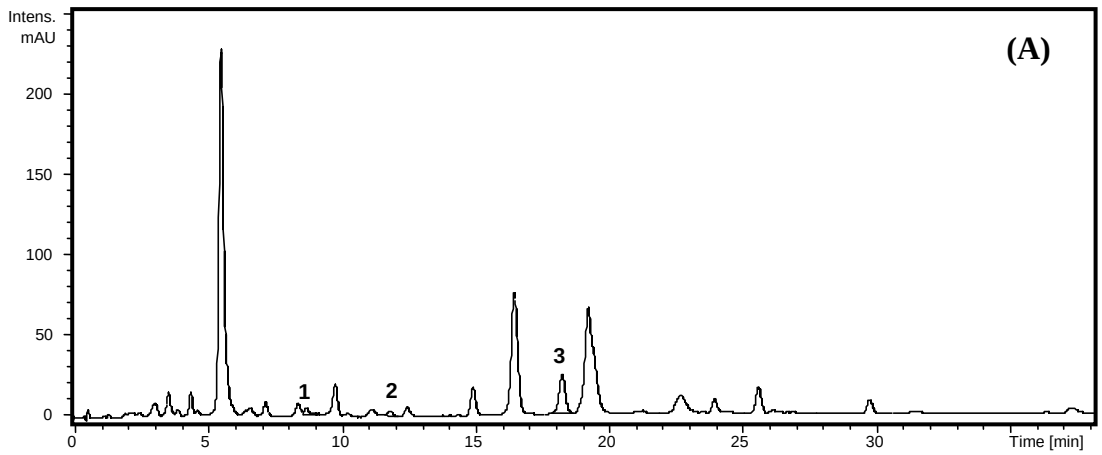
Table 1: Total phenolics and flavonoids organic fractions

Fractions	Total phenolics (mg GAE/g of fraction)	Total flavonoids (mg QE/g of fraction)
n-HF	22.367 ± 0.406^{f}	-
DCMF	329.940 ± 3.693^{b}	5.507 ± 0.372^{b}
ACNF	70.870 ± 2.142^{e}	5.247 ± 0.194^{b}
EAF	598.327 ± 4.113^{a}	22.468 ± 0.181^{a}
MeOHF	161.047 ± 1.888^{c}	0.431 ± 0.034^{c}
n-BuOHF	92.947 ± 2.732^{d}	1.294 ± 0.260^{c}

***n*-HF: *n*-hexane fraction; ACNF: acetonitrile fraction; DCMF: dichloromethane fraction; EAF: ethyl acetate fraction; *n*-BuOHF: *n*-butanol fraction. Values are mean ± SD (n = 3). Different letters in the same column indicate significant difference ($p < 0.0001$).**

The highest contents of total phenolics were detected in the EAF (598.327 mg GAE) followed by the DCMF (329.940 mg GAE). The same trend was also found with the total flavonoid contents were the EAF and the DCMF contained the high levels with 22.468 and 5.507 mg EQ, respectively. Our finding was that the total phenolics and flavonoids were significantly higher ($p < 0.0001$) in the EAF than the other fractions.

The HPLC-MS profile of this fraction has revealed the presence of one hydroxybenzoic acid, three cinnamic acid derivatives (free and ester) and three quercetol glycosides after analysis "fig. 2 (A & B)".



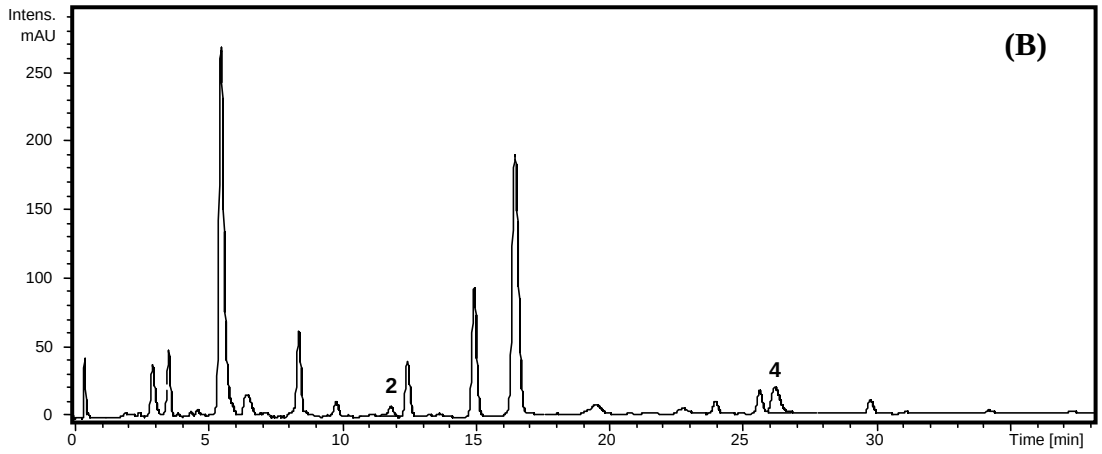


Figure 2: Polyphenols profile of ethyl acetate fraction. (A): Non Hydrolyzed fraction; (B): Hydrolyzed fraction. 1: *p*-coumaric acid; 2: Ferulic acid; 3: hyperoside; 4: Quercetol.

The *p*-coumaric acid (521.6 µg/g extract), ferulic acid (394.6 µg/g extracts) and hyperoside (7917.4 µg/g extracts) were the major compounds of the EAF "Table 2". Gentisic, caffeic and chlorogenic acids, isoquercitrin and quercitrin were also identified.

However, one aglycone of flavonol i.e. quercetol was found after hydrolysis of the EAF at a concentration of 4439.2 µg/g extracts. Considering the 18 standard compounds used in this study, several other peaks were not identified. Among these compounds, the two major peaks having retention times of 5.5 min and 16.5 min were found in the non-hydrolysed extract "fig. 2 (A & B)".

Table 2: Polyphenols contents of ethyl acetate fraction (µg/g extracts)

Polyphenols	Ethyl acetate fraction	
	NH	H
Gentisic acid	×	×
Caffeic acid	×	×
Chlorogenic acid	×	×
p-coumaric acid	521.6	×
Ferulic acid	394.6	1031.6
Hyperoside	7917.4	-
Isoquercitrin	×	-
Quercitrin	×	-
Quercetol	-	4439.2

NH: Non-hydrolyzed fraction

H: Hydrolyzed fraction

-: Not found

×: Qualitative

4. DISCUSSION

Polyphenols have gained much more attention and have become an important focus of research interest, owing to their antioxidant activities and various beneficial effects on human health, such as antioxidant, antimutagenic and anticargionogenic effects, as well as their ability to modify gene expression.[13, 14, 15] The EAF obtained from hydroacetonic extract of *I. asarifolia* contains some phenolic compounds. Chlorogenic and caffeic acids found in our study have been previously identified in aqueous extracts of the leaves.[5] However, new phenolic compounds were identified: gentisic, *p*-coumaric and ferulic acids, hyperoside, isoquercitrin, quercitrin and quercetol. Such phenolic compounds are well documented for their antioxidant properties.

Gentisic acid inhibited the LDL oxidation in a concentration-dependent manner and the formation of cholesterol ester hydroperoxides in plasma.[16] Free ferulic acid may have a positive effect on inflammation, diabetes, cancer, aging[17, 18] and may serve an important antioxidant function in preserving physiological integrity of cells exposed to both air and impinging UV radiation.[19] Hyperoside has been shown to possess various biological functions against ROS induced damage, such as the antidepressant effect by inhibiting nitric oxide synthase in rat blood and cerebral homogenate, the inhibitory effect of linoleic acid peroxidation or deoxyribose degradation induced by ROS, the partial uncoupling effect of oxidative phosphorylation in cardiac mitochondria.[20] Quercetin is considered to be a strong antioxidant due to its ability to scavenge free radicals and bind transition metal ions. These properties of quercetin allow it to inhibit lipid peroxidation.[21] One can speculate that the ethyl acetate and the dichloromethane fractions of *I. asarifolia* might decrease the lipid peroxidation by scavenging the free radicals.

Ferulic acid exhibited protective effects by reducing CCl_4-mediated oxidative stress through decreased production of free radical derivatives and attenuated hepatic glutathione depletion after CCl_4 injection.[22] This could justify the potent hepatoprotective activity of *I. asarifolia* against CCl_4-induced hepatic damage in rats.[6] Moreover, some authors have also demonstrated that hyperoside has protective effects against CCl_4-induced acute liver injury, and this protection is likely due to enhancement of the antioxidative defense system and suppression of the inflammatory response.[20, 23]

These results showed that *I asarifolia* contains some bioactive substances useful in the treatment of the diseases associated with oxidative stress, justifying the widespread uses of this species in the treatment of convulsions, rheumatism, malaria, teething children, stomach pains, ophthalmias, neuralgia, seizures and malaria in Burkina Faso folk medicines.

5. CONCLUSION

The phytochemical screening of the fractions obtained from the aqueous acetone extract indicated that *Ipomoea asarifolia* is a rich source of polyphenols. Among the fractions quantify for their total phenolic and flavonoid contents our finding was that the EAF contains the highest level. Five phenol acids (gentisic, caffeic, chlorogenic, *p*-coumaric and ferulic acids) and four flavonoids (hyperoside, quercitrin, isoquercitrin and quercetol) have been identified in this fraction after HPLC-MS analysis. The results obtained through the measurements of the antioxidant activity demonstrated that *I. asarifolia* exhibit a remarkable ferric reducing power, ABTS radical cation scavenging capacity and free radical scavenging capacity. One can notice that the nature and content of polyphenols would be responsible to this antioxidant activity.

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